Parasitic Helminths: New Perspectives in Biology and Infection

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28 August to 2 September 2022

abstracts

Poster Session 2

Wednesday 31 August 2022
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The gastrointestinal parasitic nematode genus *Strongyloides* has a unique and complex life cycle that alternates between genetically identical parasitic and free-living generations, making them a great model to study parasitism. Identification and characterisation of life cycle specific gene expression is important for understanding the fundamental principles of parasitic mechanisms at a molecular and genetic level. Previous research has shown that mRNAs are differentially expressed in the parasitic and free-living adults, implying an underlying mechanism of post-transcriptional gene regulation. However, most of our knowledge about mRNA transcripts in parasites is incomplete as it relies on short read sequencing which is often missing information about alternative splicing, 5'UTRs, 3'UTRs and polyA tail, which are associated with an important role in gene regulation. In this study, using Oxford Nanopore long-read sequencing, we have identified full-length transcripts for the gastrointestinal parasite *Strongyloides ratti* for five life cycle stages. Together, we have obtained 60 million reads with a minimum of 76% of reads representing full-length transcripts per sample. The sequenced life cycle stages of *S. ratti* include genetically identical parasitic and free-living adults, both with at least two million full-length transcripts per replicate. Using two bioinformatic approaches we have identified more than 10,000 novel transcript variants and 4000 putative novel genes across the five life cycle stages. In at least 80% and almost 50% of full-length mRNA we have established the 3'UTR or 5'UTR sequences, respectively. To better understand the mechanisms of gene regulation in parasitism, we investigate how alternatively spliced transcripts, UTRs and polyA tails vary between life cycle stages. These new data will help us recognise the role of differentially expressed transcript variants and UTR variation in parasitism and how long read sequencing can improve upon current genome annotations.

Resistance to macrocyclic lactone (ML) anthelmintic preventives in the dog heartworm *Dirofilaria immitis* is an emerging concern worldwide. Although ML-resistant isolates of *D. immitis* have been shown to be genetically distinct from wild-type populations, relatively little is known about their drug resistance mechanisms. Investigating strategies employed by the resistant parasites to circumvent the effect of MLs is crucial to identify novel molecules that could be targeted for the development of alternative therapies against heartworm disease. Therefore, it is important to determine characteristic traits of available *D. immitis* drug-resistant isolates. In this effort, we used untargeted metabolomics profiling to characterize, quantify and compare the metabolic profile of excreted/secreted products (ESPs) of drug-susceptible Missouri (MO) and drug-resistant JYD-34 isolates. The analysis of the mass spectrometry data using MetaboAnalyst 5.0 showed that PCA score plot clearly discriminated MO and JYD-34 indicating that they have distinct metabolic profiles. We found several altered metabolites between MO and JYD-34 suggesting that resistant isolates may release different biochemical compounds than susceptible isolates, which can contribute to their survival under drug pressure. Additional bioinformatics analysis showed that tryptophan and tyrosine metabolism pathways as well as drug metabolism pathway were differentially expressed between MO and JYD-34. We identified varying enrichment profiles of several metabolites linked to the differentially expressed pathways. This study would provide insights on the mechanism of drug resistance in *D. immitis* but also in other human filarial nematodes. We hope to use this knowledge to identify molecules that could be the basis for alternative therapies that aim to potentiate the effect of MLs.
Monolayers derived from equine enteroids – a new tool to study host parasite interactions

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Research into interactions between equine parasites and their host is complicated by the ethical dilemmas of animal experiments and the complex life cycle of the parasites. Stem-cell derived organoid cultures have recently emerged as a powerful tool to study these interactions in vitro. In this study, we have used monolayers of equine jejunum enteroids as an in vitro model to study the response of the intestinal epithelium to the equine parasitic helminths *Parasascaris univalens*, *Strongylus vulgaris* and Cyathostominia. Equine enteroids were dissociated and cultured as monolayers on transwell inserts in 24 well plates using growth factor-supplemented medium with or without the Th2 polarizing cytokines IL-4 and IL-13 added to the basolateral medium. Approximately 20-25 exsheeted cyathostomin L3 larvae, exsheeted *S. vulgaris* L3 larvae or hatched *P. univalens* larvae were added to the apical surface of the confluent monolayer. After 20 h of parasite exposure, the enteroid monolayers were analysed for expression of cell lineage markers (SOX9, LYZ, PCNA, EPCAM, CGA, MUC-2, DCLK1) and cytokines related to parasite infection (IL-5, IL-8, TGF-β, TSLP, IL-33) using qPCR. Preliminary results show that monolayers primed by IL-4 and IL-13 upregulated the mucin-encoding gene MUC-2, tuft cell marker gene DCKL1 and the pro inflammatory cytokine IL-8. The expression of MUC-2 was further upregulated after apical exposure to the different parasite larvae in the primed monolayers. Production of mucin by these monolayers was confirmed by immunofluorescence staining for MUC-2. These results indicate that the equine enteroid monolayers can be stimulated to reflect some aspects of the physiological weep and sweep response seen in gastrointestinal parasite infections, thus showing great potential to be used as in vitro models for future studies of host-parasite interactions.

Using *C. elegans* to study the interaction of microbiome and parasitic worms’ anaerobic metabolism

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Parasitic worms such as hookworm and whipworm infect over a billion people worldwide — they are major pathogens. With only a small number of anthelmintics available and rising resistance in multiple species, we need new strategies to treat these infections. The complex lifecycle of parasitic worms creates a unique target for treatment; once these worms infect the host’s gut, they must adapt to its low oxygen environment. To do this, parasites switch to an unusual form of anaerobic metabolism that requires the electron carrier rhodoquinone (RQ). RQ-dependent metabolism is critical for parasite survival inside the host. Crucially, RQ is not produced or used by humans. Blocking RQ synthesis or utilization would therefore kill the parasite while not affecting the host. We found that bacterial diet affects the worms RQ-dependent metabolism; to elucidate exactly how bacteria affect the RQ metabolism, we screened the Keio collection, a library of nearly 4000 single deletion *E. coli* mutants. Our results suggest bacterial iron metabolism plays a crucial role in the RQ metabolism of the worms that appears to be more complex than simple iron availability or deficiency. In particular, we found two bacterial genes, ybiL(fiu) and ybiX, which are involved in iron metabolism and whose deletions have opposite effects on the worms RQ metabolism. Studying these and similar genes shows potential not only to tell us more about nutritional requirements of the parasites in the gut, but mechanisms of uptake of iron, a major limiting micronutrient, in general. Most importantly it suggests that altering iron uptake in the gut microbiome may allow us to modify the metabolism of parasitic worms in the human gut in a way that would decrease the parasitic load. We hope that this will open up a new area for treatment of these major human pathogens.
Helminths have long been proposed as modulators of vaccine-specific responses, by impairing priming, and accelerating response waning. We hypothesised that *Schistosoma mansoni* (*Sm*) infection suppresses responses to unrelated vaccines, and that this effect can be reversed, at least in part, by intensive praziquantel treatment intervention. We conducted a randomised-controlled trial of intensive versus standard intervention against *Sm* among school children in Koome islands, Uganda.

Participants in the intensive arm received three doses of praziquantel (PZQ) (40mg/kg) each two weeks apart (the last of these 2-4 weeks before the first immunisation, BCG, at week 0), followed by quarterly during follow up. Participants in the standard arm received annual PZQ at weeks 8 and 52.

Participants received a portfolio of five licensed vaccines (BCG [week 0], Yellow Fever, oral typhoid, HPV prime [week 4], HPV booster, tetanus/diphtheria [week 28]). Data were collected at baseline and at each follow up visit; primary outcome was vaccine responses at week 8. *Sm* infection status was determined retrospectively through plasma measurement of CAA. We enrolled 478 participants, 239 in each arm. Among the *Sm* positive at baseline, preliminary data (intention to treat) indicates that intensive *Sm* treatment significantly reduced infection intensity by week 0 (median [IQR] CAA concentration, pg/ml, 29[7;226] vs 1527[317;9105] in the standard arm), and significantly improved the week 8 BCG-specific IFN-γ response as assessed by ELISpot: geometric mean ratio SFU/10^6 PBMC 1.30 (95%CI 1.03-1.64); there was no effect on the tetanus/diphtheria-specific response. Data analysis on other vaccines is underway. Our preliminary data support the hypothesis that current helminth infection reduces the response to BCG, and may have greater impact on live than inert vaccines. Our further data will contribute to the debate of whether effective control of *Sm* infection is likely to improve vaccine responses (and by extension efficacy) in endemic settings.
New accessory proteins are required for *Brugia malayi* ACR-16

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Pentameric ligand gated ion channels are biologically important protein complexes in helminths and are the targets of many anthelmintics. Channel recombination has been traditionally achieved using *Xenopus laevis* oocytes, however challenges in receptor reconstitution is common. The nicotinic acetylcholine receptor (AChR) ACR-16 is homomeric and in *X. laevis* oocytes requires the co-injection of the RIC-3 accessory protein. Our efforts to reconstitute ACR-16 from the later clade III *Brugia malayi* proved unsuccessful, in contrast to the robust responses produced from the earlier clade III *Ascaris suum* ACR-16. This suggests that specific changes occurred along the phylogeny of ACR-16 preventing its function in oocytes with only RIC-3. To identify this change, ACR-16 from intermediate species were characterized in order of phylogeny; *Dracunculus medinensis*, *Gongylonema pulchrum* and *Thelazia callipaeda*. We found smaller current responses progressively from *A. suum* to none for *B. malayi*, and this correlated with an increase in expression time. All measurable receptors show similar pharmacology suggesting little change in receptor characteristics. Together, these suggest decreased surface expression was responsible for the phylogenetic declining response. We included several accessory proteins known to be involved in receptor processing and were able to measure a functional *B. malayi* ACR-16 in combination with EMC-6, NRA-2 and NRA-4. Using these new accessory proteins, we characterized the first recombinant *B. malayi* AChR. EMC-6, NRA-2 and NRA-4 function in subunit folding and assembly within the ER, it is likely that this process has changed within later clade III worm receptors. By using a novel approach of phylogeny receptor characterization, here we provide the first *B. malayi* AChR ex vivo characterization, propose an explanation for the difficulty in producing *B. malayi* AChRs, and provide novel insight into the evolutionary relationship between receptors and their regulation machinery.

MicroRNAs (miRNAs) are small non-coding RNAs that can regulate gene expression post transcriptionally. Identification of miRNAs in parasitic helminth excretory-secretory (ES) products and extracellular vesicles (EV) has led to interest in these small RNAs as potential modulators of host responses to infection. Parasite miRNAs have been shown to alter levels of host gene expression, suggesting they may regulate host genes to benefit parasite survival. Using bioinformatic and experimental approaches, we are investigating potential host target transcripts of miRNAs that are enriched in ES products and EV of *Haemonchus contortus*, a prevalent blood-feeding gastrointestinal nematode of ruminants, related to hookworms. miRNA target prediction from ovine and bovine genome data was performed using three different algorithms (miRanda, RNAhybrid and TargetSacan) for the ten most abundant miRNAs in *H. contortus* L4 and adult ES and EV. The results from the three algorithms were integrated, and the location of predicted miRNA binding sites in the mRNA 3’UTRs and the site type were defined. *In silico* functional analysis of predicted miRNA targets was performed and identified genes involved in gastrointestinal immune regulation, epithelial stem cells and epithelial cell homeostasis, among others. Expression of selected putative target genes in the ovine abomasum was identified from RNA-seq data and confirmed by RT-qPCR. We are focusing on potential miRNA regulation of two predicted targets: ovine CD69 (mucosal immune gene) and KLF4 (gut-enriched Krüppel-like factor 4). miRNA-mRNA interactions are being tested by luciferase reporter-3’UTR assays. In addition, we have established ovine abomasum organoids and are using these to examine effects of specific miRNA mimics and EVs on gene expression and epithelial cell phenotypes following miRNA or EV exposure. Our findings will help define molecular mechanisms that parasites may use to modulate infection outcome in the GI tract.
Global look at *Schistosoma mansoni* egg gene expression: differences among three strains and eggs from intestine vs. liver

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The eggs of the blood fluke *Schistosoma mansoni* are the main cause of the pathological manifestations of schistosomiasis. Adult worms reproduce in the mesenteric veins where they lay eggs which then either migrate from the blood vessel across the intestinal wall into the gut lumen or are carried away and become tissue-entrapped, typically in the liver. During these processes, eggs secrete molecules that interact with host blood, and tissues, and modulate immunity. Our study aims to identify essential egg-derived proteins involved in this interplay. We applied RNA-sequencing and bioinformatic analysis to investigate gene-expression dynamics among specifically defined egg samples. First, we compared immature (in blood vessel) and mature (tissue entrapped) eggs to describe the transcription changes during the migration of the egg. Next, we examined differences between eggs isolated from the intestine vs. from the liver, as it is known that the character of the granulomatous immune response varies between those two organs. Lastly, we are currently analyzing eggs originating from three different laboratory strains: Puerto Rican, Brazilian and Liberian to determine what genes may be responsible for a distinct pathological manifestation of the defined mouse strain. This comprehensive approach revealed distinct transcription patterns for various protein groups. Based on the findings of our ongoing studies, we revealed previously neglected molecules that are most likely important for the egg-host interaction and could help to further clarify sophisticated egg biology.

Neurotoxocarosis – meta-analysis of tropisms, pathomechanisms, transcriptomics and lipidomics associated with neuroinvasive *Toxocara* larvae

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Neurotoxocarosis (NT) is a syndrome caused by neuroinvasive third stage larvae (neural larva migrans, NLM) of *Toxocara canis* or *Toxocara cati* migrating through and persisting in the central nervous system of paratenic hosts where they can cause severe neurological symptoms. The knowledge about NT is comparably limited, wherefore several researchers, including our group, focused on the investigation of NLM to improve our understanding of *Toxocara*-induced neuropathology. In the past decade, we unravelled several defined aspects of NLM in the mouse, being the most appropriate model organism for NT. Amongst others, we could show the differential neurotropism of *T. canis* and *T. cati*, *Toxocara*-mediated neuronal demyelination, neurobehavioural as well as memory function deficits and pathological, transcriptional as well as lipidomic changes in infected mice. All of these studies were performed under similar conditions, including the same mouse (C57Bl/6JR) and parasite (HannoverTcanis2008, HannoverTcati2010) strains, infective doses (2000 embryonated eggs) and examination time points (14, 28, 42, 70 and 98 days *post infectionem*), making these data suitable for comparative meta-analysis. Thus, above-mentioned findings will be investigated for correlations during the course of infection in a model-based approach. In conclusion, this meta-analysis will provide a holistic and comprehensive overview on and unravel the relation between tropisms, pathomechanisms, transcriptomics and lipidomics associated with neuroinvasive *Toxocara* larvae.
Obesity has become a major global health care challenge. It is associated with a chronic low-grade pro-inflammatory state both locally in the adipose tissue and systemically, affecting peripheral immune responses. While it has been previously shown that helminth infection can ameliorate obesity and improve metabolic health, it is currently unclear how obesity affects peripheral type 2-biased immune responses as well as the function of ILC2, eosinophils, and Th2 cells. Due to the shift towards systemic Th1/Th17-inflammation, we hypothesize that obesity impairs peripheral immunity against helminths.

In order to investigate the impact of obesity on type 2 immune responses, we infected mice that have been kept on HFD with the gastrointestinal helminth *Nippostrongylus brasiliensis* and studied the ensuing pulmonary Th2-polarized inflammatory response. We analysed innate effectors, such as eosinophils, neutrophils, macrophages and ILC2, as well as adaptive T cell responses including their polarization and activation state. Furthermore, we investigated how antibody production is affected by obesity, which may interfere with immunity against challenge infections. We observed that infection of obese animals significantly increased neutrophilic inflammation in the lung. Additionally, we found significantly decreased eosinophil numbers. Interestingly, these findings were dependent on the sex of the infected animal. Furthermore, ILC2 numbers in the lungs of female, but not male mice were affected by obesity and IL-13 production was impaired. Similarly, Th2 cell polarization in the lung was impaired and obese animals had a higher worm burden following infection with *N. brasiliensis*. Taken together, obesity alters pulmonary type 2 responses leading to increased susceptibility to helminth infection in a sex-dependent manner. The interference with Th2 cell function may have important consequences for secondary infections and vaccinations, and extend to type 2-related immune responses including atopic dermatitis and asthma.
Antischistosomal activity of snake venoms against *Schistosoma mansoni* schistosomula and adult worms


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Schistosomiasis is a neglected tropical disease affecting more than 200 million people worldwide. Controlling the disease is still a challenge, as treatment is restricted to a single drug which does not act on juvenile worms, with reports of parasite tolerance. Therefore, discovery of new compounds for treatment of schistosomiasis is needed. Natural products have been suggested as possible sources of active compounds against helminths, including *Schistosoma mansoni*. Among these, snake venoms contain numerous bioactive components, although they have been little explored against schistosomes. In this work, we analyzed for the first time the effects of the in vitro treatment of *S. mansoni* with venoms of eight snakes from the genus *Bothrops* and from *Crotalus durissus terrificus*. Viability was followed at different venom concentrations on adult worms for up to 72h and on schistosomula for up to 120h. A dose and time-dependent effect from all tested venoms on the reduction of viability was observed in both adults and schistosomula. Venoms’ ability to decrease *S. mansoni* viability varied, highlighting their diverse qualitative and quantitative compositions. All venoms killed adult worms at 50 µg/mL after 72h in culture, with strong inhibition of motility, pairing and oviposition at earlier timepoints. *S. mansoni* schistosomula viability was completely abolished by five out of the nine tested venoms at 6.25 µg/mL after 24h in culture. A low correlation of venoms’ potencies in adults and schistosomula was observed, suggesting different targets in both stages. RT-qPCR assays showed that genes related to parasite feeding and digestion are affected by the venoms in schistosomula. The observed antischistosomal activity suggests that components of *Bothrops* and *Crotalus d. terrificus* venoms should be investigated in more detail as potential antischistosomal and anthelmintic leads. Further tests with venoms fractions will pave the way for the discovery of snake toxins with anthelmintic potential.
The type 2 immune response is a hallmark of asthma and allergy but also host defence against helminth parasite infection. The nematode *Heligmosomoides polygyrus* secretes multiple immunomodulatory proteins, notably: HpARI and HpBARI which antagonises the IL-33 pathways, HpTGM which mediates a regulatory response by inducing Treg cells. All three molecules are structurally similar consisting of a string of consecutive atypical Complement Control Protein (CCP) domains. This led to the hypothesis that CCP domain-containing proteins may represent a family of immunomodulatory molecules used by *H. polygyrus* to evade the host immune response. Protein domain prediction tools do not classify HpARI and HpTGM as containing CCP domains, therefore bioinformatic sequence analyses was carried out to develop an atypical CCP motif. To identify CCP domain-containing proteins like the *H. polygyrus* immunomodulators, this new motif was screened against *H. polygyrus* genomic and transcriptomic data. Candidates were selected based on features of CCP domain and were produced as recombinant proteins using the Expi293 mammalian expression system and finally assessed for protein-protein interaction using the Avidity-based Extracellular Interaction Screening (AVEXIS) assay. Here, we have identified 93 potential CCP domain-containing proteins, from which 30 candidates were selected for expression as “baits”. A selection of type 2 immune targets: chemokines, cytokines and their receptors were expressed as “preys”. This assay is a sensitive system and has now been optimised to show robust interactions between HpARI:IL-33, HpBARI:ST2, and HpTGM:TGF-β receptor. We are now screening for novel protein-protein interactions between parasite molecules and immune targets. The methods and analyses described here may assist the development of a larger pipeline in identifying, producing and characterising new helminth immunomodulatory molecules. Furthermore, the AVEXIS assay can be modified as an immunoassay to assess compounds for interaction between cytokine-bait and receptor-prey directly.

*Heligmosomoides polygyrus* is a mouse specific gastrointestinal dwelling parasite whose secreted products can interfere with IL-33 signalling. *H. polygyrus* secretes HpARI, which blocks IL-33, and HpBARI, which blocks ST2 (the IL-33 receptor). With these two molecules both being shown in vitro to block the IL-33 pathway, we wanted to know how important they are to the parasite’s invasion of its host in the early stages of infection.

Without transgenic *H. polygyrus* techniques developed as yet we are utilising vaccination and monoclonal/nanobody blocking activities to help answer this question. Using in vitro selection of a camelid nanobody library, we have selected anti-HpARI and anti-HpBARI reagents for detection of these proteins in vivo. Conventional murine monoclonal antibody production was used to develop multiple anti-HpARI antibodies which block different facets of the HpARI’s activity, allowing us to further probe this host-parasite interaction. Finally, HpARI and HpBARI have been administered in vivo in a vaccination regime using an alum adjuvant, and have shown to provide sterilising immunity against the parasite. Complete protection has never been previously shown for recombinant protein vaccines in this infection, providing strong evidence for their importance during infection, and a rationale for further immunomodulatory protein vaccine design.
The porcine roundworm *Ascaris suum* leads to reduced feed conversion and weight gain, but the causative mechanisms remain incompletely understood. Therefore, changes in metabolite profiles were assessed in intestinal content and serum samples of *A. suum*-infected pigs by nuclear magnetic resonance spectroscopy. Experimental groups comprised pigs infected once with 10,000 *A. suum* eggs as well as pigs that received a trickle-infection (1,000 eggs/day over ten days) and a non-infected control group. Six pigs each per group were sacrificed on days 21, 35 and 49 post infection (pi). Overall, trickle-infected pigs showed more pronounced metabolic changes than single-infected pigs. On day 21 pi, a significant increase of the short-chain fatty acids (SCFAs) acetate and butyrate as well as certain amino acids (tyrosine, proline) was noted in the colon of trickle-infected as compared to control animals. The opposite pattern was evident on day 35 pi, with significantly reduced concentrations of SCFAs in the caecum and colon. Less pronounced changes were observed on day 49 pi. Significant changes in the single-infected group included an increase of lactate and acetate in the ileum, and of certain amino acids in the caecum on day 35 pi. Analysis of serum samples revealed, among others, lower glucose levels on day 21 and 35 pi, and an increase of acetate and butyrate on day 49 pi in the trickle-infected group. The observed alterations may be related to *A. suum*-induced changes in intestinal transport physiology and to altered patterns of intestinal microbiota composition. With regard to the latter, a synthesis of metabolomic and bacterial 16S rRNA sequencing data will be presented.

Schistosomiasis affects over 230 million people worldwide, with varied, stage specific morbidity. A better understanding of the interaction between the parasite and its human host could provide opportunities for preventing infection and/or disease. Here, we use a controlled human *Schistosoma mansoni* infection to comprehensively map early immune responses in schistosomiasis. Using unbiased, high dimensional immunological techniques we were able to reveal an early inflammatory response, characterized by an expansion of CD38+ monocytes and effector memory T cells, which was paralleled by an expansion of Th2 type cytokine response. Following this, at week 8 post infection, we have observed an expansion of regulatory cell subsets, including CD11c+ atypical memory B cells and IL-10 producing CD4-CD8- T cells. Alongside these regulatory responses, we saw a reduction in expression of the pro-inflammatory cytokine TNF at 8 weeks. Taken together, the controlled human schistosome infection model indicates that upon infection, a strong inflammatory response is followed by the onset of regulatory responses, possibly to control tissue damage. Moreover, combining the infection with in depth profiling of immune cells, a number of cell clusters that have not been reported before, were shown to be involved in response of human host to *Schistosoma mansoni*. 

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While extensive studies have provided an understanding of the molecular mediators in the mammalian host invasion by *Fasciola hepatica*, little is known on the interaction with the intermediate host. It is particularly interesting since two or more cycles of asexual amplification occur within lymnaeid snails, leading to the production of hundreds of infective metacercariae. We analyzed transcriptomic data from miracidia and intra-molluscan stages seeking for clues of these interaction and developmental processes. We found evidence for the expression of 1744 novel transcripts and several isoforms of annotated genes. Comparing expression across the whole cycle by diverse methods resulted in five distinct groups of gene expression (egg, miracidia, intra-snail stages, invading stages and juvenile-adults). Few genes showed strict stage-specific expression, but notably most of those corresponding to miracidial and intra-snail stages are novel unannotated genes. Genes upregulated in miracidial stages include enzymes involved in neurotransmitter synthesis, energy metabolism and calcium mediated signaling, consistent with the needs of the short-lived free-living stage. Stage specific SCP/TAPS proteins and trypsin-like proteases were also detected in miracidia. Several genes associated with development and morphogenesis are characterized in early (15 dpi) intra-snail stages. Purine salvage pathway genes are upregulated in this stage, consistent the high biosynthetic needs and the absence of a complete purine synthesis pathway in *F.hepatica*. Mucins, glycan biosynthesis genes and aquaporins upregulated within late (30 dpi) intra-snail stages are interesting, considering their putative role in the following host transition. Notably, stage specific expression of several members of well-known protein families involved in host interaction such as cathepsin proteases, legumains, protease inhibitors, lipid transporters and SCP/TAPS proteins can be detected both in early and late intra-snail stages. These results highlight that the parasite might have tuned during evolution the same set of molecular mediators for the specific interaction with the intermediate and definitive hosts.
Lipids are of vital importance in biology of parasitic worms, particularly in relation to cellular membranes, energy storage, and intra- and intercellular signalling. Despite the recent expansion of the lipidomics field, there had been no comprehensive investigation of the lipidome of any parasitic nematodes. Here, using high-performance LC-MS/MS, we characterised the global lipidome of *Haemonchus contortus*. We identified and quantified hundreds of lipid species from six developmental stages [eggs; third-stage, exsheathed L3s and fourth-stage larvae; female and male adults] and two organ systems [reproductive and alimentary tracts] of adult female *H. contortus*. We observed substantial alterations in the lipid composition and abundance, and assigned key roles in cellular processes and functions (e.g. energy storage regulation and membrane structure) to the distinct stages and organ systems. The findings suggest that the nature and extent of lipids are linked to stage of growth and development as well as a need to adapt to constantly changing environments within and outside of the host animal. This lipidomic dataset serves as a stimulus for studies to understand lipid biology in parasitic nematodes, and their roles in parasite-host interactions and disease processes.

PS3 is a widely studied tumor suppressor that is most famous for being mutated in over 50 percent of human malignancies. Despite its well-characterized role as a tumor suppressor, the ancestral function of PS3 is somewhat unclear as it is found across a variety of metazoans, notably in invertebrates that do not develop malignancies. The prevailing theory for why this is the case is that PS3 originally evolved to protect the germline of early metazoans from genotoxic stress such as UV radiation by inducing cell cycle arrest and apoptosis. In this study, we examine the function of PS3 homologs in Platyhelminthes by studying PS3 homologs in the free-living planarian flatworm *Schmidtea mediterranea* and the parasitic flatworm *Schistosoma mansoni*. Each worm possesses a PS3 homolog that is orthologous to canonical PS3, and these orthologs regulate stem cell maintenance and differentiation, but not the response to genotoxic stress. Unlike planarians, schistosomes possess a parasite-specific PS3 paralog that is required for the parasite’s normal response to genotoxic stress. This suggests that the ability to respond to genotoxic stress in parasitic flatworms may have arisen from convergent evolution of a tumor suppressor-like function in a PS3 homolog.
Opisthorchis felineus is one of the three most medically important species belonging to the family of fish borne zoonotic trematodes known as Opisthorchiidae. O. felineus is endemic to the river plains of Western Siberia and Eastern Europe and it is estimated that more than 1.6 million people could be infected with these parasites. Chronic opisthorchiasis may lead to severe hepatobiliary morbidity and an elevated risk of cholangiocarcinoma (bile duct cancer) which is an aggressive cancer with a 5-year survival rate of 25% when diagnosed at an early stage. O. felineus adult flukes were collected from experimentally infected hamsters and cultured in vitro in serum free media. We extracted proteins from different compartments of the O. felineus secretome, including (i) soluble excretory/secretory (ES) products (depleted of extracellular vesicles by ultracentrifugation); (ii) secreted microvesicles; and (iii) tegument. The tegument was further separated into surface, membrane and intravesicular compartments via sequential extraction. Using a combination of RNAseq transcriptomics, high-resolution mass spectrometry and SDS-PAGE separation and protein digestion, we identified 686, 894, 389, 324 and 165 proteins from the ES, microvesicular and the three different tegument protein compartments respectively. We further performed in-depth gene ontology and protein family analyses on the identified proteins as well as comparisons against a similar proteome dataset acquired from the SE Asian liver fluke Opisthorchis viverinni. Identifying and characterizing proteins using transcriptomics and proteomics approaches is more biologically informative and meaningful than using genomics alone. The information from this study will form a biologically relevant dataset of O. felineus proteins that could be used in developing diagnostic and therapeutic tools to manage the human cost of O. felineus infections and its associated diseases.